EXHIBIT 3

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Regulation of γ-Glutamyl-Cysteine Synthetase by Nonallosteric Feedback Inhibition by Glutathione*

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SUMMARY

γ-Glutamyl-cysteine synthetase is inhibited by glutathione under conditions similar to those which prevail *in vivo*, thus strongly suggesting a physiologically significant feedback mechanism. Inhibition by glutathione, which is not allosteric, appears to involve the binding of glutathione to the glutamate site of the enzyme as well as to another enzyme site; the latter binding appears to require a sulfhydryl group since ophthalmic acid (γ-glutamyl-α-aminobutyryl-glycine) is only a weak inhibitor. The finding that glutathione regulates its own synthesis by inhibiting synthesis of γ-glutamyl-cysteine appears to explain observations on patients with 5-oxoprolinuria, who were shown to have a block in the γ-glutamyl cycle consisting of a marked deficiency of glutathione synthetase and consequently of glutathione. These patients produce greater than normal amounts of γ-glutamyl-cysteine, which is converted by the action of γ-glutamyl cyclotransferase to 5-oxoproline; production of the latter compound exceeds the capacity of 5-oxoprolinase to convert it to glutamate. The apparent K_m value for L-cysteine for γ-glutamyl-cysteine synthetase (0.35 mM) is not far from intracellular concentrations of L-cysteine suggesting that the availability of L-cysteine may also play a role in the regulation of glutathione synthesis.

Current information indicates that glutathione is synthesized from its constituent amino acids in virtually all living cells. The biosynthesis of glutathione is catalyzed by two enzymes, first demonstrated by Snoke and Bloch (1),[1] which catalyze Reactions 1 and 2, respectively, where M^{2+} = Mg^{2+} or Mn^{2+}.

L-Glutamate + L-Cysteine + ATP

$$\xrightarrow[\text{synthetase}]{M^{2+};\ \gamma\text{-glutamyl-cysteine}} \quad (1)$$

L-γ-glutamyl-L-cysteine + ADP + P_i

L-γ-Glutamyl-L-cysteine + glycine + ATP

$$\xrightarrow[\text{synthetase}]{M^{2+};\ \text{glutathione}} \quad (2)$$

glutathione + ADP + P_i

Previous studies in this laboratory on glutathione synthetase provided evidence for the intermediate formation of an acyl phosphate in the reaction catalyzed by this enzyme (3, 4) and also led to other related findings on an apparently homogeneous preparation of this enzyme (4–6). Earlier work had indicated that the reaction catalyzed by glutamine synthetase also involves an acyl phosphate intermediate, *i.e.* γ-glutamyl phosphate (7, 8), and more recent studies on γ-glutamyl-cysteine synthetase indicate that γ-glutamyl phosphate is also an intermediate in this reaction (9, 10). Ammonia is not a substrate for γ-glutamyl-cysteine synthetase, nor is L-cysteine a substrate for glutamine synthetase; nevertheless, both enzymes interact effectively with a specific diastereoisomer of methionine sulfoximine (L-methionine-*S*-sulfoximine) leading to marked inhibition associated with binding to the respective enzymes of L-methionine-*S*-sulfoximine phosphate (2, 8, 10–14). Although the reactions catalyzed by glutamine synthetase and γ-glutamyl-cysteine synthetase are thus similar, it is notable that glutamine synthetases from a variety of sources are multi-subunit enzymes (8, 15), while γ-glutamyl-cysteine synthetase is not.[2]

The biosynthesis of glutathione has been studied with enzyme preparations obtained from various sources including liver, kidney, erythrocytes, *Escherichia coli*, and certain plants (2). In general, the catalytic capacity of cells to synthesize glutathione (as measured *in vitro* under optimal conditions) seems to exceed the apparent over-all turnover of the intracellular glutathione. The concentrations of glutathione in various cells (*e.g.* erythrocytes, liver, kidney) appear to be relatively constant under specific conditions, although there are reports indicating that intracellular concentrations of glutathione can be affected by various conditions including starvation, neoplasia, anaesthesia, and stress (16–20).

It seems reasonable to suppose that, like other biosynthetic pathways, the synthesis of glutathione is controlled, and indeed it has been suggested that erythrocyte (21, 22) and liver (23) glutathione may exert a regulatory effect on its own synthesis. In the present work, we have examined the effect of glutathione and of several related compounds on the activity of kidney γ-glutamyl-cysteine synthetase. This work was stimulated by

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[1] This subject has been reviewed recently (2).

[2] Unpublished data (1974).

recent observations in this laboratory which showed that patients with the inborn error of metabolism 5-oxoprolinuria (pyroglutamic aciduria) exhibit a marked generalized deficiency of glutathione synthetase, and apparently also increased γ-glutamylcysteine synthesis (24). The findings reported here indicate that glutathione effectively inhibits the synthesis of γ-glutamylcysteine under conditions which seem to approximate those which occur *in vivo*. In contrast to certain feedback inhibitory phenomena (25–27), the effect of glutathione is not allosteric.

EXPERIMENTAL PROCEDURES

Materials—Glutathione, glutathione disulfide, *S*-methylglutathione, 2-mercaptoethanol, L-α-aminobutyric acid, disodium ATP, dithiothreitol, dithiothreitol disulfide, 5,5′-dithiobis(2-nitrobenzoic acid), semicarbazide hydrochloride, carboxypeptidase A, and bovine serum albumin were obtained from Sigma. L-Glutamic acid and disodium ethylenediaminetetraacetic acid were obtained from Schwarz-Mann, L-glutamine was obtained from Calbiochem, L-γ-glutamylhydrazide was obtained from Nutritional Biochemicals, L-cysteine was obtained from Mann, L-α-aminobutyrylglycine was obtained from Bachem, and *o*-phthalaldehyde was obtained from Aldrich. The γ-glutamyl hydrazones of pyruvic acid, α-ketobutyric acid, α-keto-*n*-valeric acid, α-keto-*n*-caproic acid, and α-ketocaprylic acid were the products previously described (28). L-γ-Glutamyl-L-α-aminobutyric acid was prepared as described (29, 30). Di-L-γ-glutamyl-L-cysteine was prepared by the action of carboxypeptidase A on glutathione disulfide according to the method of Strumeyer and Bloch (31).

Ophthalmic acid was prepared by incubating rat kidney γ-glutamyl transpeptidase with glutathione and L-α-aminobutyrylglycine as follows. Glutathione (0.576 g, 1.88 mmol) and L-α-aminobutyryl-glycine (1.0 g, 0.25 mmol) were dissolved in water and the pH was adjusted to 8.0 by adding sodium hydroxide; the final volume was brought to 100 ml with water, and 1 ml of purified γ-glutamyl transpeptidase (19.2 units)[2] was added (32). The solution was incubated at 37° for 2 hours. The product was isolated by chromatography on a column of Dowex 1-acetate (X8, 200 to 400 mesh, 4 × 46 cm) prepared as described by Cohen (33). The reaction mixture was added (at 5°) to the top of the column, which was then washed with 4 liters of water. Elution was carried out with 2 liters each of acetic acid solutions of the following concentrations: 0.05, 0.10, 0.20, 0.30, and 0.50 M. Ophthalmic acid eluted with 0.50 M acetic acid. The fractions containing the product were combined and flash-evaporated (below 40°) to low volume. This solution was lyophilized to a white powder (180 mg); m.p. 138–140° (uncorrected). The product did not give a color reaction with 5,5′-dithiobis(2-nitrobenzoic acid) (34). When the product was chromatographed on the amino acid analyzer it emerged as a single peak with the same retention time as aspartic acid. After acid hydrolysis (6 N HCl, 115°, 48 hours) the product gave glutamic acid, α-aminobutyric acid, and glycine in equimolar quantities as determined on the amino acid analyzer.

$C_{11}H_{19}O_4N_3 2H_2O$

Calculated: C 40.61, H 7.08, N 12.92
Found: C 40.15, H 7.41, N 12.85

γ-Glutamyl-cysteine synthetase was isolated from rat kidney by the method previously described (35) except that after Step 4, the pooled fractions from the DEAE-cellulose column were concentrated in an Amicon ultrafiltration cell equipped with an XM-50 filter. After concentration to 5 to 10 mg of protein per ml, the enzyme was dialyzed at 5° against Tris-HCl buffer (50 mM, pH 8.2) and then stored at 0°.

Methods—Enzyme activity was determined essentially as described (35). The standard assay reaction mixtures contained Tris-HCl buffer (100 mM, pH 8.2), sodium L-glutamate (10 mM), L-α-aminobutyrate (10 mM), magnesium chloride (20 mM), disodium ATP (5 mM), disodium ethylenediaminetetraacetic acid (2 mM), bovine serum albumin (40 μg per ml), and enzyme in a final volume of 0.50 ml. After incubation at 37° for 2 to 30 min, the reaction was terminated by adding 0.50 ml of 10% (w/v) trichloroacetic acid. The inorganic phosphate released was determined by the method of Fiske and SubbaRow (36). The intensity of the blue color obtained was determined at 720 nm and compared to that of appropriate blanks (*e.g.* a reaction mixture lacking enzyme). One unit of enzyme activity is defined as the amount that catalyzes the release of 1 μmol of orthophosphate per hour under the conditions described above. The inclusion of low concentrations of ethylenediaminetetraacetic acid (2 mM) and bovine serum albumin (40 μg per ml) in the assay mixtures was found necessary for optimal activity.

In the experiments with L-cysteine and γ-glutamyl-cysteine all solutions were deaerated by vacuum and the incubations were carried out under nitrogen in stoppered tubes.

The reduction of di-γ-glutamyl-cystine to γ-glutamyl-cysteine was accomplished with dithiothreitol. The amount of dithiothreitol required to reduce completely a given amount of di-γ-glutamyl-cystine was determined by assaying for γ-glutamylcysteine formation after adding various amounts of dithiothreitol. The γ-glutamyl-cysteine formed was determined by an adaptation of the fluorescence assay procedure for glutathione described by Cohen and Lyle (37). γ-Glutamyl-cysteine, like glutathione, forms a complex with *o*-phthalaldehyde that fluoresces intensely at 420 nm when excited at 350 nm.[4] Di-γ-Glutamyl-cystine (1 to 2.5 μmol) was dissolved in 0.25 ml of a solution (deaerated under vacuum and maintained under nitrogen) containing Tris-HCl buffer (200 mM, pH 8.2), sodium L-glutamate (4 mM), L-α-aminobutyrate (4 mM), magnesium chloride (8 mM), disodium ATP (2 mM), disodium ethylenediaminetetraacetic acid (0.8 mM), and bovine serum albumin (80 μg per ml). Dithiothreitol in 20% molar excess was then added and the solutions were incubated under nitrogen for 30 min at 37°. Di-γ-Glutamyl-cystine is completely reduced under these conditions.

Amino acid analyses were performed on a Durrum amino acid analyzer.

RESULTS

Inhibition of γ-Glutamyl-cysteine Synthetase by Glutathione—When the enzyme was assayed by the procedure given under "Methods," but in the presence of added 5 and 10 mM glutathione, its activity was inhibited by 22 and 31%, respectively. Addition of glutathione led to virtually identical inhibition when the assays were carried out in reaction mixtures in which L-α-aminobutyrate was replaced by L-cysteine. When the inhibition by glutathione was examined as a function of L-glutamate concentration, the results obtained showed that glutathione is a competitive inhibitor with respect to L-glutamate (Fig. 1). The apparent K_i value for glutathione calculated from these data was 2.3 mM. Data currently available on the content of glutathione and L-glutamate in rodent kidney indicate that these compounds are present in concentrations which are in the range of about 2 to 4 mmoles per kg of tissue (38). The apparent K_i value for glutathione is not far from values for the concentration of glutathione in rat kidney which have been found in recent studies in this laboratory (2.44 ± 0.27).[5] Experiments were also carried out in which concentrations of L-α-aminobutyrate, L-glutamate, Mg^{2+}, and ATP were used which are lower than those used in the standard assay system. Thus, in the studies shown in Fig. 2, the time course of the reaction was followed in the presence and absence of two different concentrations of glutathione. In these experiments the concentration of L-glutamate was 2 mM, thus approximating the physiological concentration of this amino acid. Substantial inhibition was observed in the presence of glutathione; no inhibition was observed with 10 mM 2-mercaptoethanol (*Curve B*).

The experiments shown in Fig. 3 (*A* and *B*) show that the inhibition of γ-glutamyl-cysteine synthetase produced by 10 mM glutathione (under conditions similar to those used in the study

[2] We are indebted to Dr. S. S. Tate of this laboratory for providing generous amounts of the transpeptidase.

[4] R. Sekura, unpublished observations in this laboratory.

[5] A. Palekar, unpublished data obtained in this laboratory.

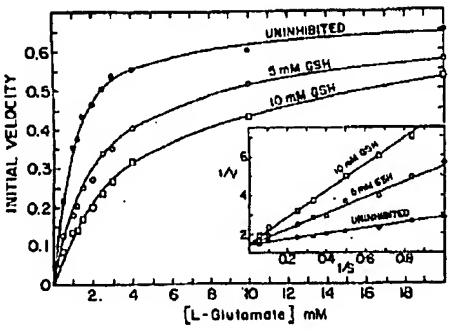

FIG. 1. Effect of L-glutamate on inhibition by glutathione. The reaction mixtures contained sodium L-glutamate (0.5 to 20 mM), Tris-HCl buffer (100 mM, pH 8.2), disodium ATP (5 mM), $MgCl_2$ (20 mM), L-α-aminobutyrate (10 mM), disodium ethylenediaminetetraacetic acid (2 mM), bovine serum albumin (40 μg per ml), enzyme (1.1 units), and the indicated concentrations of glutathione in a final volume of 0.50 ml, all incubated for 20 min at 37°. The inorganic phosphate formed was determined as described under "Methods;" initial velocity is expressed in terms of the $\Delta A_{720\ m\mu}$ observed. The *inset* gives the double reciprocal plot of the data.

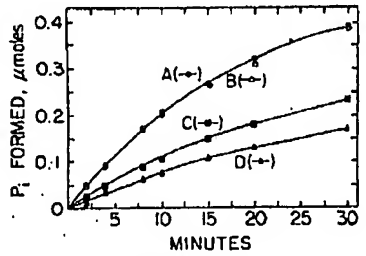

FIG. 2. Time course of the reaction at low substrate concentrations in the presence and absence of glutathione. The enzyme (58 units) was incubated at 37° in a final volume of 5.5 ml containing Tris-HCl buffer (100 mM, pH 8.2), sodium L-glutamate (2 mM), L-α-aminobutyrate (2 mM), $MgCl_2$ (4 mM), disodium ATP (1 mM), disodium ethylenediaminetetraacetic acid (0.4 mM), bovine serum albumin (40 μg per ml), and the following compounds: *Curve A*, none (control); *Curve B*, 10 mM 2-mercaptoethanol; *Curve C*, 5 mM glutathione; *Curve D*, 10 mM glutathione. At the indicated intervals, 0.50-ml aliquots were removed and added to 0.50 ml of 10% (w/v) trichloroacetic acid. The formation of P_i was then determined as described under "Methods."

described in Fig. 2) can be overcome by increasing the concentration of L-glutamate (Fig. 3*A*), but when the concentration of L-α-aminobutyrate was increased (Fig. 3*B*) the inhibition was not substantially reduced. Similarly, as shown in Fig. 3*D*, the inhibition by glutathione was not reduced by increasing the concentration of L-cysteine.

In previous studies on this enzyme in our laboratory, L-α-aminobutyrate was used as the amino acid acceptor rather than L-cysteine because of the marked tendency of the latter amino acid to undergo spontaneous oxidation to the disulfide. However, under appropriate conditions (see "Methods"), valid data can be obtained with L-cysteine in systems in which oxygen is largely excluded and in which relatively short periods of incubation are used. Using such conditions, the apparent K_m value for L-cysteine was determined (Fig. 3*C*); a value of 0.35 mM was obtained from these data. This value is substantially lower than that obtained (3 mM) for L-α-aminobutyrate in analogous studies on the same enzyme preparation carried out under these conditions.

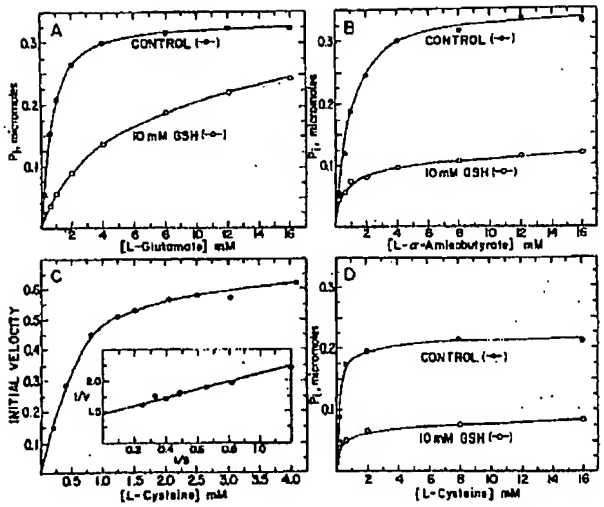

FIG. 3. Factors affecting γ-glutamyl-cysteine synthetase activity. *A*, effect of L-glutamate on inhibition by glutathione; *B*, effect of L-α-aminobutyrate on inhibition by glutathione; *C*, effect of L-cysteine concentration on activity; *D*, effect of L-cysteine on inhibition by glutathione. In *A*, *B*, and *D*, the reaction mixtures (final volume, 0.50 ml) contained Tris-HCl buffer (100 mM, pH 8.2), disodium ATP (1 mM), $MgCl_2$ (4 mM), disodium ethylenediaminetetraacetic acid (0.4 mM), bovine serum albumin (40 μg per ml), enzyme (12 units), 10 mM glutathione as indicated, and, in *A*, L-α-aminobutyrate (2 mM) and L-glutamate as indicated, in *B* and *D*, sodium L-glutamate (2 mM) and either L-cysteine or L-α-aminobutyrate as indicated; all incubated for 10 min at 37° in *A* and *B*, and for 5 min in *D*. The inorganic phosphate released was determined as described under "Methods." In *C*, the reaction mixtures contained L-cysteine as indicated, Tris-HCl buffer (100 mM, pH 8.2), disodium ATP (5 mM), $MgCl_2$ (20 mM), sodium L-glutamate (10 mM), disodium ethylenediaminetetraacetic acid (2 mM), bovine serum albumin (40 μg per ml), and enzyme (3.2 units) in a final volume of 0.50 ml; incubated 8 min at 37°. The inorganic phosphate released was determined as described under "Methods;" initial velocity is expressed in terms of $\Delta A_{720\ m\mu}$. The *inset* gives the double reciprocal plot of the data.

Studies with Compounds Structurally Related to Glutathione—The data given in Fig. 4 summarize studies on the effects of several compounds that are structurally related to glutathione on γ-glutamyl-cysteine synthetase activity. Of the various compounds examined, only L-γ-glutamyl-L-cysteine proved to be an effective inhibitor. It is notable that ophthalmic acid (L-γ-glutamyl-L-α-aminobutyryl-glycine) was much less effective in inhibiting the reaction than was glutathione; similarly, L-γ-glutamyl-L-α-aminobutyrate also produced much less inhibition than did L-γ-glutamyl-L-cysteine. These findings seem to reflect the importance of the sulfhydryl group for inhibition. Consistent with this idea is the finding that glutathione disulfide and *S*-methylglutathione are also relatively poor inhibitors of the enzyme.

The γ-glutamyl moiety is evidently also needed for inhibition since 2-mercaptoethanol and dithiothreitol did not inhibit. In the course of this work several additional γ-glutamyl compounds were studied; the most interesting of those thus far examined proved to be the L-γ-glutamyl hydrazones of certain α-keto acids. These compounds were originally prepared and studied in connection with their ability to serve as substrates for liver glutamine transaminase (type L) (28). When γ-glutamyl-cysteine synthetase activity was determined in the standard assay system (see "Methods") in the presence of 10 mM L-γ-

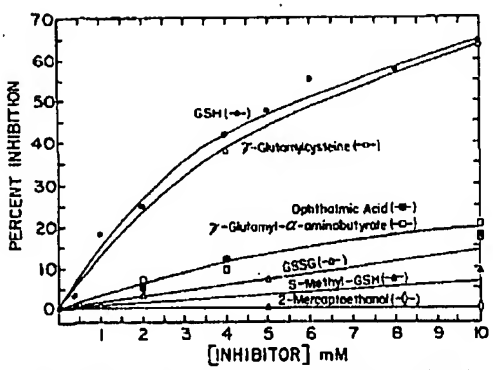

FIG. 4. Inhibition by compounds structurally related to glutathione. The reaction mixtures (final volume, 0.5 ml) contained Tris-HCl buffer (100 mM, pH 8.2), sodium ATP (1 mM), $MgCl_2$ (4 mM), sodium L-glutamate (2 mM), L-α-aminobutyrate (2 mM), disodium ethylenediaminetetraacetic acid (0.4 mM), bovine serum albumin (40 μg per ml), enzyme (7.5 units), and the indicated compound (10 mM); incubated for 20 min at 37°. The inorganic phosphate formed was determined as described under "Methods."

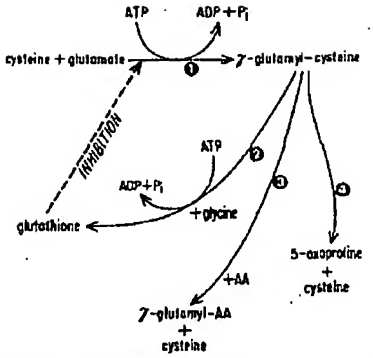

FIG. 5. Metabolic interrelationships involved in glutathione synthesis (see text): *1*, γ-glutamyl-cysteine synthetase; *2*, glutathione synthetase; *3*, γ-glutamyl transpeptidase; and *4*, γ-glutamyl cyclotransferase.

glutamyl hydrazones of α-keto acids, substantial inhibition was observed with certain derivatives. Thus, the L-γ-glutamyl hydrazone derivatives of pyruvate, α-ketobutyrate, α-keto-*n*-valerate, α-keto-*n*-caproate, and α-keto-*n*-caprylate inhibited the reaction by 5, 41, 50, 39, and 0%, respectively. Under these conditions, L-γ-glutamyl hydrazide, L-glutamine, α-ketobutyrate, and α-ketobutyrate semicarbazone did not inhibit.

DISCUSSION

The findings reported here show that glutathione produces substantial inhibition of γ-glutamyl-cysteine synthetase activity under conditions which are similar to those which probably occur *in vivo*. Such inhibition by glutathione thus seems to reflect a physiologically significant feedback phenomenon. The data indicate that inhibition is not allosteric and indeed that glutathione evidently binds to the glutamate site of the enzyme, rather than to a separate site. The observation that γ-glutamyl-α-aminobutyrate and ophthalmic acid inhibit much less than the corresponding sulfhydryl compounds suggests that glutathione also binds to another site on the enzyme, and that its sulfhydryl group is involved in such binding. The data are not inconsistent with the idea that glutathione binds both to the glutamate and cysteine sites of the enzyme. Indeed, the relatively low inhibitions observed with γ-glutamyl-α-aminobutyrate and ophthalmic acid as compared to the inhibitions found with the corresponding sulfhydryl analogs seems to correlate closely with the relative affinities of L-α-aminobutyrate and L-cysteine (as judged by their respective apparent K_m values). Another possibility that must be considered is that the binding of glutamate (or of the γ-glutamyl moiety of glutathione) to the glutamate site produces a conformational change in the enzyme which facilitates binding of cysteine (or of the cysteinyl moiety of glutathione) to the cysteine site. It should be noted, however, that the enzyme can evidently bind cysteine and α-aminobutyrate in the absence of glutamate, since these amino acids were found to activate the ATPase activity of the enzyme (9). Further studies on the interaction of the enzyme with its several substrates are needed to elucidate fully these various binding phenomena.

The experiments reported here are clearly relevant to previous studies in this laboratory on tissues obtained from patients with the inborn error of metabolism, 5-oxoprolinuria. It was shown that patients with this disease exhibit markedly decreased intracellular concentrations of glutathione. Studies on the γ-glutamyl cycle enzymes in erythrocytes, cultured skin fibroblasts, and placenta obtained from these patients showed that the metabolic lesion in this disease is at the glutathione synthetase step of the γ-glutamyl cycle (24). Thus, the glutathione synthetase activity of the placenta was about 2% of that of a control, while the activity of the cultured skin fibroblasts was less than 5% of that of controls. A marked deficiency of glutathione synthetase activity was also found in the erythrocytes from the patients; the values were between 5 and 10% of controls. These data were interpreted to indicate that the 5-oxoprolinuria exhibited by these patients is secondary to glutathione synthetase deficiency. Thus, a deficiency of glutathione synthetase would lead to 5-oxoprolinuria if much more than normal amounts of γ-glutamyl-cysteine were formed and converted to 5-oxoproline, and if such overproduction of 5-oxoproline exceeded the capacity of 5-oxoprolinase to convert it to glutamate. It was suggested previously that γ-glutamyl-cysteine may normally be protected from the action of γ-glutamyl cyclotransferase (Fig. 5, *Reaction 4*), perhaps by close linkage between the two synthetases or by compartmentalization within the cell (39). It was also noted that γ-glutamyl-cysteine is a good substrate for γ-glutamyl transpeptidase (32) and might therefore serve in place of glutathione in the γ-glutamyl cycle in transpeptidation reactions with amino acids (24) (Fig. 5, *Reaction 3*). However, in contrast to glutathione, γ-glutamyl-cysteine is an excellent substrate of γ-glutamyl cyclotransferase, and one would therefore expect that γ-glutamyl-cysteine not used for glutathione synthesis or for transpeptidation reactions would be rapidly converted to 5-oxoproline and cysteine (Fig. 5, *Reaction 4*). The previous suggestion that there is an overproduction of γ-glutamyl-cysteine in 5-oxoprolinuria leading to a futile cycle of γ-glutamyl-cysteine synthesis followed by its conversion to 5-oxoproline and cysteine is in accord with the present finding that glutathione regulates its own synthesis by inhibition of γ-glutamyl-cysteine synthesis. Thus, in 5-oxoprolinuria, the marked reduction of glutathione synthetase and consequent decrease of intracellular glutathione leads to increased γ-glutamyl-cysteine synthesis. The present findings thus offer additional insight into the biochemical mechanisms involved in this inborn error of metabolism and also into the mechanisms that normally control glutathione synthesis.

It is highly probable that the rate of synthesis of glutathione is also influenced by other metabolic phenomena, and the present work suggests that the intracellular concentration of cysteine

may be one of these. The apparent K_m value for L-cysteine found here (0.35 mM), which is in fair agreement with values obtained in studies on γ-glutamyl-cysteine synthetase preparations from erythrocytes (21, 40) and lens (41), is probably not far from the usual intracellular concentrations of cysteine. Thus, a value of 0.045 mM (as cystine) was reported for cat kidney (42). It thus seems possible that the availability of cysteine for γ-glutamyl-cysteine synthesis could play a significant role in the regulation of glutathione synthesis. This possibility was suggested also by Jackson (21) in relation to glutathione synthesis in the erythrocyte. It is evident that factors that affect the rate of synthesis or utilization of cysteine by other metabolic reactions, as well as those that can influence the interconversion of cystine and cysteine may also play a role in the regulation of glutathione synthesis.

REFERENCES

1. Snoke, J. E., and Bloch, K. (1954) in *Glutathione-A Symposium* (Colowick, S., Lazarow, A., Racker, E., Schwarz, D. R., Stadtman, E., and Waelsch, H., eds) pp. 129–141, Academic Press, New York
2. Meister, A. (1974) in *The Enzymes* (Boyer, P., ed) Vol. 10, (3rd Ed), pp. 671–697, Academic Press, New York
3. Nishimura, J. S., Dodd, E. A., and Meister, A. (1963) *Fed. Proc.* 22, 536
4. Nishimura, J. S., Dodd, E. A., and Meister, A. (1963) *J. Biol. Chem.* 238, PC1179–1180
5. Nishimura, J. S., Dodd, E. A., and Meister, A. (1964) *J. Biol. Chem.* 239, 2553–2558
6. Mooz, E. D., and Meister, A. (1967) *Biochemistry* 6, 1722–1734
7. Krishnaswamy, P. R., Pamiljans, V., and Meister, A. (1962) *J. Biol. Chem.* 237, 2932–2940
8. Meister, A. (1974) in *The Enzymes* (Boyer, P., ed) Vol. 10, (3rd Ed), pp. 699–754, Academic Press, New York
9. Orlowski, M., and Meister, A. (1971) *J. Biol. Chem.* 246, 7095–7105
10. Richman, P. G., Orlowski, M., and Meister, A. (1973) *J. Biol. Chem.* 248, 6684–6690
11. Ronzio, R., and Meister, A. (1968) *Proc. Natl. Acad. Sci. U.S.A.* 59, 164–170
12. Ronzio, R. A., Rowe, W. B., and Meister, A. (1969) *Biochemistry* 8, 1066–1075
13. Rowe, W. B., Ronzio, R. A., and Meister, A. (1969) *Biochemistry* 8, 2674–2680
14. Manning, J. M., Moore, S., Rowe, W. B., and Meister, A. (1969) *Biochemistry* 8, 2681–2685
15. Stadtman, E. R., and Ginsburg, A. (1974) in *The Enzymes* (Boyer, P., ed) Vol. 10, pp. 755–808, Academic Press, New York
16. Edwards, S., and Westerfeld, W. W. (1952) *Proc. Soc. Exp. Biol. Med.* 79, 57–59
17. Beck, L. V., Rieck, V. D., and Duncan, B. (1958) *Proc. Soc. Exp. Biol. Med.* 97, 229–231
18. Elwyn, D. H., Parikh, H. C., and Shoemaker, W. C. (1968) *Am. J. Physiol.* 215, 1260–1275
19. Jocelyn, P. C. (1969) in *Glutathione* (Crooke, E. M., ed) pp. 43–65, Cambridge University Press
20. Bartlett, R. G., Jr., and Register, U. D. (1955) *Proc. Soc. Exp. Biol. Med.* 90, 500–502
21. Jackson, R. C. (1969) *Biochem. J.* 111, 309–315
22. Wendel, A. (1974) in *Glutathione* (Flohé, L., Benohr, H. Ch., Sies, H., Waller, H. D., and Wendel, A., eds) pp. 69–78, Georg Thieme, Stuttgart
23. Davis, J. S., Balinsky, J. B., Harlington, J. S., and Shepherd, J. B. (1973) *Biochem. J.* 133, 667–678
24. Wellner, V. P., Sekura, R., Meister, A., and Larsson, A. (1974) *Proc. Natl. Acad. Sci. U.S.A.* 71, 2505–2509
25. Umbarger, H. E. (1956) *Science* 123, 848
26. Yates, R. A., and Pardee, A. B. (1956) *J. Biol. Chem.* 221, 757–770
27. Monod, J., Changeux, J., and Jacob, F. (1963) *J. Mol. Biol.* 6, 306–329
28. Cooper, A. J. L., and Meister, A. (1973) *J. Biol. Chem.* 248, 8489–8498
29. Le Quesne, N. J., and Young, G. T. (1950) *J. Chem. Soc.* 1954–1959, 1959–1963
30. Sachs, H., and Brand, E. (1954) *J. Am. Chem. Soc.* 76, 1815–1817
31. Strumeyer, D., and Bloch, K. (1962) in *Biochemical Preparations* (Coon, M. J., ed) Vol. 9, pp. 52–54, John Wiley and Sons, New York
32. Tate, S. S., and Meister, A. (1974) *J. Biol. Chem.* 249, 7593–7602
33. Cohen, W. E. (1957) *Meth. Enzymol.* 3, 724–743
34. Ellman, G. L. (1959) *Arch. Biochem. Biophys.* 82, 70–77
35. Orlowski, M., and Meister, A. (1971) *Biochemistry* 10, 372–380
36. Fiske, C. H., and SubbaRow, Y. (1925) *J. Biol. Chem.* 66, 375–400
37. Cohen, V. H., and Lyle, J. (1966) *Anal. Biochem.* 14, 434–440
38. Sekura, R., and Meister, A. (1974) *Proc. Natl. Acad. Sci. U.S.A.* 71, 2969–2972
39. Meister, A. (1973) *Science* 180, 33–39
40. Majerus, P. W., Brauner, M. J., Smith, M. B., and Minnich, V. (1971) *J. Clin. Invest.* 50, 1637–1643
41. Rathbun, W. B. (1967) *Arch. Biochem. Biophys.* 122, 62–72
42. Tallan, H. H., Moore, S., and Stein, W. H. (1954) *J. Biol. Chem.* 211, 927–939